

Policy & Procedure (P&P)

Policy Title :

Indirect Antiglobulin Test (IAT) (or Indirect Coombs or The antibody screening)

Department	Index No.	Scope
Laboratory & Blood Bank	LAB-072	BLOOD BANK STAFF
Issue Date	Revision NO	Effective Date
14/10/1432	3	1441/7/19
Review Due Date	Related Standard NO.	Page Number#
1443/7/19	CBAHI (61)	8

01. Policy:

IAT test is done to demonstrate all clinically unexpected antibodies in a blood sample

02. Definition :

02.1. N/A

03. Purpose :

1. Indirect antiglobulin test will be performed on all samples received with a request for transfusion, and when especially requested.
2. In accordance to AABB, samples will be tested by a method that will demonstrate all clinically unexpected antibodies.
3. Pre-transfusion testing will be added to all AHG tests interpreted as negative.
4. IgG sensitized red cells will be added to all AHG tests interpreted as negative.
5. The sample must be obtained from the patient within 3 days of the scheduled transfusion.

04. Procedure :

The indirect antiglobulin test is performed by incubating patient serum with reagent screening red cells(1,2&3) for approximately 20 minutes and then observing for agglutination. If the antibody screen is positive, additional testing is required to determine the specificity of the antibody.

The indirect antiglobulin test is useful for:

1. Cross-match (patient serum is incubated with donor red cells).
2. Antibody screen (patient serum is incubated with group O screen cells 1,2 & 3).
3. Antibody identification (patient serum is incubated with group O panel cells).

Note: The following are possible reasons why this test may be done:

- 1) ABO incompatibility reaction.
- 2) Rh incompatibility reaction.

4. Method

Indirect antiglobulin (Coombs) test can be done by any one of the following technique:

- A) Tube method.
- B) Gel Microtyping System.
- C) TANGO Optimo System technique

A) Tube Method

1. Specimen Requirements

- 1.1. Fresh serum obtained from fully clotted specimens should be used in indirect antiglobulin test procedures. The use of plasma is not recommended since anticoagulants may interfere with the detection of complement binding antibodies. Fibrin clots may also interfere. Serum should be separated from the red cells immediately and testing should be performed as soon as possible. If testing is delayed, the serum should be stored at 2-8°C for no more than 48 hours or frozen.
- 1.2. Hemolyzed specimens are unacceptable for indirect antiglobulin test procedures because some antigen antibody reactions are detected by visible hemolysis in the test tube.

2. Materials

- 2.1. Glass test tubes
- 2.2. Disposable Blood Bank pipettes
- 2.3. Calibrated serologic centrifuge
- 2.4. Isotonic normal saline 0.85 NaCl
- 2.5. Microscope
- 2.6. 37°C water bath
- 2.7. Automatic cell washer

3. Reagent

- 3.1. Antibody screen cells (1,11&111)
- 3.2. 22% bovine albumin
- 3.3. Coombs reagent
- 3.4. Coombs control cells

4. Steps

- 4.1. Verify that patient information on the sample matches information on the test request.
- 4.2. Bring the three vials of antibody screen to room temperature and label 3 test tubes.
- 4.3. Add one drop of (O) cell suspension from each vial to the corresponding labeled tube.
- 4.4. Add 1-2 drops of patient serum to each of the 3 labeled tubes and gently mix, read and record results.
- 4.5. Add 2 drops of 22% bovine albumin.
- 4.6. Incubated at 37°C for 15-30 minutes, centrifuge, resuspend, read and record results.
- 4.7. Washed well 3-4 times to remove all unbound protein.
- 4.8. Add 2 drops of AHG serum and gently mix
- 4.9. Centrifuge for 15 seconds at 3400 rpm.
- 4.10. Examine for agglutination.
- 4.11. Add one drop Coombs control cells to any tubes which have been recorded as negative.
- 4.12. Record results in the log book.

5. Quality Control

Reagents are to be tested with appropriate positive and negative controls daily and the results recorded. See "Daily Quality Control" for procedure.

6. Reporting Results

6.1. Interpretation

- 6.1.1. No agglutination indicates a negative indirect antiglobulin test(there is no antibodies in the serum, capable to reacting with the test cells).
- 6.1.2. Agglutination indicates a positive indirect antiglobulin test(presence of antibodies in the serum, capable to reacting with the test cells).
- 6.1.3. In case of discrepancy between screening cell results and cross matching results, one of the following antibodies may be responsible:



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6.1.3.1. Anti-H in A1 and A1B individuals may agglutinate (O) and A2 cells.

6.1.3.2. Anti L3bh react only with OLeb+ cells but not with A1 or A1B that are Leb+.

6.1.3.3. AntiA1 in A2 individuals will not react with O cells (Screening cells) but will react with A1 cells during cross matching.

6.1.3.4. Low incidence antigens.

6.1.3.5. Antigens on homozygous cells only react with the corresponding antibody.

6.1.4. In false negative results we may use:

6.1.4.1. Enzymes as ficin panel.

6.1.4.2. Adjustment of incubation time to not more than 30 minutes with 22% bovine albumin.

6.1.4.3. Equal amount of serum and cells with 22% bovine albumin.

6.2. Record results immediately

6.3. Repeat any test that is negative with CCC

6.4. Do not increase serum to cell with bovine albumin

7. Limitations

The washing stage is critical, because if any unbound protein (IgG) remains, it could neutralize the anti-human globulin. Also residual saline after the last wash will dilute the anti-human globulin serum.

B) Indirect Coombs Test using Column Technology and the Gel Microtyping System

1. Materials

1.1. ID-Centrifuge

1.2. ID-Incubator 37°C

1.3. ID-Working table

1.4. ID-Dispenser

1.5. ID-Pipette EP-3/EP-4/FP-3

1.6. ID-Disposable tips

1.7. ID-Suspension tubes

2. Reagents

2.1. ID-Diluent 2 (modified LISS)

2.2. ID-Dia Cell I, II, III (Antibody screen cell)



3. Microtyping Cards
LISS/Coombs

4. Sample
Serum or Plasma.

5. Test Procedures

- 6.1. Allow all reagents to reach room temperature before use.
- 6.2. Identify the ID-micro typing card with the patient name and number and remove the aluminum foil.
- 6.3. Add 50µl of ID-DiaCell I, II, III to micro tube 1, 2, 3.
- 6.4. Add 25µl of test serum or plasma to each micro tubes.
- 6.5. Incubate the micro typing card for 15 minutes at 37°C in the ID-incubator.
- 6.6. Centrifuge the micro typing card for 10 minutes in the ID-centrifuge.

6. Interpret the result

- 7.1. Positive reaction, grade 1-4 in the micro tube indicates the presence of an antibody. Further investigation using an antibody identification panel by the appropriate technique, will be required to confirm this.
- 7.2. Negative reaction in the micro tubes indicate the absence of an antibody.

7. Quality Control

Reagents and microtubes are to be tested with appropriate positive and negative controls daily (ID-Internal Quality Control) and the results recorded. See "Daily Quality Control" for procedure.

C) Indirect Coombs Test using TANGO Infinity System (Fully Automated)

1. Principle of the test

Solidscreen™ II Strip plates are composed of test strips (12 strips with 8 wells each) that have been coated with Protein A.

Protein A has a high binding affinity for the Fc region of immunoglobulins.

The method used for the Direct Coombs test on the Solidscreen™ II Strip is solid phase adherence. If red blood cell alloantibodies and/or autoantibodies are present in the sample to be tested they will bind to the patients red blood cells.

Unbound antibody is removed by washing.

After adding Anti-Human- Globulin specially formulated for the Solidscreen™ II assay and centrifugation Anti-Human-Globulin forms a link between the protein A on the surface of the



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microwell and the patients red blood cells. Thereby a layer of red blood cells will form on the surface of the microwell.

But if no red blood cell alloantibodies or autoantibodies are present in the sample, the cells will not get coated with antibodies and therefore will not form a cell layer on the surface of the microwell but sediment to the bottom of the well to form a cell button.

2. Sample

Serum or plasma after proper centrifugation.

3. Reagents

2.1. **Solidscreen™ II Strip Plates** with 12 strips per plate. The strips are coated with protein A.

2.2. **MLB 2 Modified LISS 2 (Low Ionic Strength Solution)** is an antibody enhancement media specifically formulated for use with the Solidscreen™ II test system. The MLB 2 is used in the preparation of a 1% cell suspension of sample red blood cells.

2.3. **Alsevers Solution** Used as a suspension medium for red blood cells.

2.4. **Anti-Human Globulin (Anti-IgG Solidscreen™ II)** The Anti-Human Globulin is used to demonstrate the presence of antibodies bound to the donors red blood cell that do not cause direct agglutination.

4. Materials

3.1. Glass test tubes

3.2. (N) rack of the tango infinity machine

3.3. Calibrated serologic centrifuge

5. Test Procedures

5.1. Centrifuge Patients blood sample & put them in (N) Rack.

5.2. Open the sample door & put the (N) Rack in its correct place.

5.3. Push the Rack button to select the desired rack on the screen.

5.4. Press manual entry button & write the ID number of patients sample.

5.5. Press accepted entry button.

5.6. Press (ABSd3) button to select the test & close the sample door.

5.7. Press start button to start the test.

5.8. After the end of the test validate the results.

5.9. Print out the results.

5.10. Record the results in the special logbook.



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6. Quality Control

Control Set (QC) contain test erythrocytes with defined antigen pattern for ABO, Rh (D), phenotyping {Rh (CEce) and Kell} as well as isoagglutinins for reverse typing.

6.1. Group O Neg

6.2. Group AB Pos

6.3. Group A Neg

6.4. Group O Pos

6.5. Solidscreen 11-Control is used for Antibody screen QC.

05. Responsibilities :

05.1. All Blood Bank Staff of Al-Qunfudah General Hospital.

06. Equipment & Forms

Donor notification form

07. Attachment :

07.1. N/A

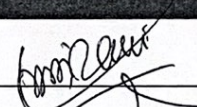
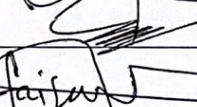
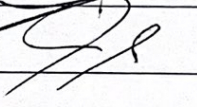
08. Reference

AABB technical manual 18th edition, 2016

The unified practical procedure manual for blood banks in the arab countries 2013

TANGO INFINITY MANUAL

Preparation, Reviewing & Approval Box

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